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CLEMATOSIDE C - TRITERPENIC OLIGOSIDE FROM CLEMATIS MANSHURICA

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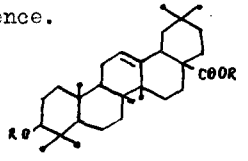
We have isolated from Clematis manshurica Rupr. roots a triterponic oligoside named clematoside C.[1] Now we suggest full structure I for this compound on the basis of the following evidence.



I R = L-Rha 1→6 D→Gl 1→4 D-Gl 1→4 D-Xy 1→2 L-Ar 1→4 L-Rha
R′= L-Rha 1→4 D-Gl 1→4 D-Gl 1→6 D-Gl-1

II R = R′=H

III R = L-Rha 1→6 D-Gl 1→4 D-Gl 1→4 D-Xy 1→2 L-Ar 1→4 L-Rha
R′= H

XV R = L-Rha R′= H

Acid hydrolysis of clematoside C affords oleanolic acid (II), five moles of glucose, three moles of rhamnose and one mole of each xylose and arabinose. Oleanolic acid being the genin of clematoside C, two carbohydrate chains might

be expected by analogy with other oligosides,e.g. araloside B[2], gypsoside[3]. Ethereal CH_2N_2 does not react with clematoside C thus suggesting the presence of a carbohydrate chain bound to the carboxyl of oleanolic acid. This conclusion is supported by alkaline hydrolysis of I (5% NaOH, 90°, 6 hrs in N_2) affording glycoside (III), $[\alpha]_D^{20}$-20° (c 0.8 in CH_3OH) (Found: C 57.76, H 7.80 ; Calcd. for $C_{64}H_{104}O_{29}$: C 57.44, H 7.78%) by acyl-glycosidic bond rupture. III cintains two moles of each glucose and rhamnose and one mole of each xylose and arabinose. At the same time,oligosaccharide is formed yielding glucose and rhamnose on acid hydrolysis.

Periodate oxydation of clematoside C leads to destruction of all of the monosaccharides thus indicating the absence of 1→3 linkages as well as the absence of branching in carbohydrate chains.

Methylation according to Kuhn[4] followed by 8-9 methylations according to Purdie[5] afforded permethyl-clematoside C (IV), $[\alpha]_D^{20}$-22.7° (c 4.4 in $CHCl_3$). Found: C 58.54, H 8.38 ; Calcd. for $C_{115}H_{198}O_{48}$: C 58.82, H 8.38%. Methanolysis and subsequent hydrolysis of IV resulted in a mixture of methylated monosaccharides. Partition chromatigraphy on cellulose using successive elution with n-heptane - n-butanol (4:1), saturated with water, then with the same system but with solvents ratia 3:1 and 2:1, and then with n-heptane - n-butanol (1:1), half-saturated with water, afforded chromatographically pure 2,3,4-tri-O-methyl-L-rhamnose (V),3,4-di-O-methyl-L-arabinose (VI) and a fraction of mixed methylated monosaccharides. The latter was resolved further by

adsorbtion chromatography on charcoal - celite 535 (1:1) using aqueous methyl ethyl ketone of concentration increased from 1.5 to 5.5%. Individual 2,3,4-tri-O-methyl-D-glucose (VII), 2,3-di-O-methyl-L-rhamnose (VIII) and a mixture of 2,3-di-O-methyl-D-xylose (IX) and 2,3,6-tri-O-methyl-D-glucose (X) were obtained. The two latter monosaccharides were transformed to methyl furanosides and resolved by gas-liquid chromatography. Quantitative determination demonstrated the presence of three moles of X, two moles of each V and VII and one of each VIII, IX and VI. This result was in support of the presence in clematoside C of two sugar chains terminating with rhamnosyl residues.

Reduction of IV with $LiAlH_4$ gave rise to a glycoside which appeared erythrodiol hexaoside (XI), $[\alpha]_D^{20}$ -15.4° (c 3.6 in CH_3OH). Found: C 61.77, H 9.11 ; Calcd. for $C_{79}H_{136}O_{28}$: C 61.83, H 8.87%. At the same time, reduced tetrasaccharide (XII) was formed, $[\alpha]_D^{20}$ +6° (c 10 in CH_3OH). Found: C 52.46, H 8.28 ; Calcd. for $C_{36}H_{68}O_{20}$: C 52.68, H 8.28%. The latter afforded two moles of X and one mole of each VIII and 2,3,4-tri-O-methyl-D-sorbitol on acid hydrolysis. This proves that the oleanolic acid carboxyl in clematoside C is bound to a tetrasaccharide of composition L-Rha 1→4 D-Gl 1→4 D-Gl 1→6 D-Gl-1 (XIII). XI afforded one mole of each of the methylated monosaccharides on acid hydrolysis.

Acetylation of III with acetic anhydride in pyridine gave rise to acetate (XIV), $[\alpha]_D^{20}$ +2.7° (c3.1 in $CHCl_3$). Found: C 57.84, H 6.84 ; Calcd. for $C_{94}H_{134}O_{44}$: C 57.84, H 6.81%.

XIV was subjected to acetolysis (2% H_2SO_4 in acetic anhydride, 72°, 30 min.) and the products deacetylated with 10% $(C_2H_5)_3N$ in dry methanol (12 hrs at 25°). Paper chromatography in n-butanol - water - ethanol 10:5:2 revealed that oligosaccharides with R_{Gl} 0.77, 0.50 and 0.19 were present in the reaction mixture along with monosaccharides.

Glucose and rhamnose were identified as components of the R_{Gl} 0.77 oligosaccharide, isolated by paper chromatography. Reduction and subsequent hydrolysis of this compound afforded rhamnose and sorbitol. The oligosaccharide was identical with authentic rutinose.

The R_{Gl} 0.50 oligosaccharide afforded rhamnose and glucose on acid hydrolysis and rhamnose, glucose and sorbitol on reduction followed by hydrolysis, thus suggesting the structure L-Rha 1→6 D-Gl 1→4 D-Gl1.

The oligosaccharide with R_{Gl} 0.19 afforded rhamnose, glucose and xylose on hydrolysis and rhamnose, glucose and xylitol on reduction followed by hydrolysis, thus suggesting the sequence L-Rha 1→6 D-Gl 1→4 D-Gl 1→4 D-Xyl.

Oxydation of III with lead tetraacetate (98% CH_3COOH) for 30 min. and reduction of the product obtained with $NaBH_4$ followed by hydrolysis (0.1 N HCl, 60°, 2 hrs)[6] afforded a mixture, which contained II, III and the new glycoside (XV) as revealed by thin-layer chromatography on silicagel in n-butanol-water-ethanol 10:5:2. XV was isolated using the preparative thin-layer chromatography, it contained II and rhamnose. Thus, a rhamnosyl residue is directly attached to the C(3)-hydroxyl in II.

Hence, the above data indicate that the carbohydrate chain L-Rha 1-6 D-Gl 1-4 D-Gl 1-4 D-Xy 1-2 L-Ar 1-4 L-Rha-1 is bound with the C(3)-hydroxyl, and that clematoside C has full structure I.

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